IN VITRO ACTIVATION OF THE ENZYMIC ACTIVITY OF HEPATIC LIPASE BY apoA-II

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1. Introduction

Human post-heparin plasma contains two major lipase activities designated lipoprotein lipase (LPL) and hepatic lipase (HL) [1–3]. LPL is activated by apoC-II [4,5] and apoH (β_2 glycoprotein I) [6]. Type I hyperlipoproteinemia is associated with a deficiency in the enzymic activity of LPL [7]. A deficiency in apoC-II also results in type I hyperlipoproteinemia [8] and LPL and apoC-II have therefore been proposed to be of major importance in modulating the catabolism of triglyceride-rich lipoproteins in man. The function, natural substrate and regulation of the enzymic activity of HL have been less well defined. HL activity in human post-heparin plasma is decreased in patients with liver disease and renal failure [9–11]. An inverse correlation between HL enzymic activity and the concentration of high density lipoproteins (HDL, $d = 1.063$–1.21 g/ml [12]) as well as HDL_2 ($d = 1.063$–1.125 g/ml) has been reported and it has been suggested that HL may play a role in the catabolism of HDL_2 [13]. HL enzymic activity has been reported to be inhibited by apolipoproteins C-I, C-II and C-III, and increased by high concentrations of NaCl and serum [14]. In initial studies we have also observed that human plasma activates HL enzymic activity. It was the purpose of this study to further define the component within plasma which is responsible for this activation. The results of our investigations indicate that under the conditions studied, apoA-II, a protein constituent of human HDL activates HL.

2. Materials and methods

2.1. *Enzyme source*

Plasma was obtained from normal fasting subjects following intravenous injection of sodium heparin (75 U/kg body wt, beef lung heparin, Upjohn Co.). Blood was collected 15 min after injection, mixed with 0.25 M sodium citrate (0.05 ml/ml blood, J. T. Baker Chemical Co.) and centrifuged at 2000 rev./min for 30 min in a Sorvall RC-3 centrifuge (Dupont Instr.) at 4°C. The plasma was aspirated and stored at −70°C until used.

HL was isolated from human post heparin plasma by affinity chromatography on heparin–Sepharose CL-6B (Pharmacia Fine Chemicals). The heparin affinity column (Isolab, QS-6E, 0.6 × 1.75 cm) was equilibrated with buffer A (5 mM barbital buffer, 0.4 M NaCl, pH 7.4) and 2 ml post-heparin plasma diluted 1:2 with buffer A were applied to the column. The column was developed with 8 ml buffer A, and hepatic lipase eluted with 1 ml buffer B (5 mM barbital buffer, 0.7 M NaCl, pH 7.4). The column was washed with 8 ml buffer B and lipoprotein lipase was eluted with 1 ml buffer C (5 mM barbital buffer, 1.5 M NaCl, pH 7.4). Purified enzymes were stored at −70°C if not used immediately.

2.2. *Substrate*

9,10(*n*)-[^{3}H]glycerol triolate (triolein), 121 Ci/mmol, (New England Nuclear) in hexane and unlabeled triolein (Applied Science Labs. Inc.) were repurified by column chromatography using silicic acid (Mallinckrodt, 100 mesh) and Florisil (Fisher, 60–100 mesh) [15]. Petroleum ether (Burdick and Jackson Labs. Inc.) and anhydrous ethyl ether (Mallinckrodt) were used for column chromatography. Anhydrous emulsions of [3H]triolein in glycerol (J. T. Baker Chemical Co.) were prepared as in [15] using lecithin from egg yolk (Sigma) as stabilizer. Assay substrate [7.2 mM [^{3}H]triolein (5×10^3 dpm/nmol triolein), 2% bovine serum albumin (BSA) (Sigma fraction V), 0.13 M Tris–HCl (pH 8.6)] were prepared daily by dilution of anhydrous emulsions with 0.3 M Tris–HCl (pH 8.6) containing 5% BSA.

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2.3. *Assay systems*

Assays of HL and LPL activity were performed in 2 mM [^{3}H]triolein, 87 mM Tris–HCl, 2% BSA, 75 mM NaCl, 50 mM ammonium bicarbonate, 0.25 mM barbital buffer (final pH 8.6) in 200 μl; 10 μl enzyme preparation were assayed each time. The amount of protein or volume of plasma with different experiments is indicated in the figure legend. The incubations were initiated by addition of enzyme. The incubations were carried out for 30 min at 37°C in a shaking waterbath. The reaction was terminated and the assay products analyzed by the method in [16]. The assay was linear during the time course of these experiments and over the range of the enzyme concentrations used.

2.4. *Purification of apolipoproteins*

ApoA-II and apoC-II were purified as in [17,18]. ApoA-II was dissolved in 6 M guanidine, 0.01 M Tris, 0.1 M NaCl (pH 7.4) and dialyzed against 0.01 M ammonium bicarbonate (pH 8.6). Protein was determined as in [19].

3. Results and discussion

In these studies we investigated the effect of plasma, apoA-II and apoC-II and HL enzymic activity. The addition of normal plasma (20 μl/200 μl assay volume) increased HL enzymic activity by 240 ± 60%. Isolated apoA-II increased HL activity by 361 ± 96% under these assay conditions. A representative curve of HL enzymic activity as a function of increasing concentrations of apoA-II is shown in fig.1a. Activation occurred at low protein concentrations and reached a plateau at 20 μg protein/ml; based on M_r = 17 380 this value corresponds to 1.2 μM apoA-II. In some experiments at higher concentrations of apoA-II (>100 μg/ml), the degree of activation declined.

Increasing concentrations of apoC-II in this assay system inhibited HL enzymic activity, with >80% inhibition observed at 10 μM apoC-II (fig.1c). Apoc-II at 10 μg/ml increased LPL activity >10-fold (fig.1d). The activation of LPL by apoC-II was partially reversed by increasing concentrations of apoA-II (fig.1b). It is important to note that all of these effects occur: (i) at μM levels; and (ii) in the presence of a 200-fold greater concentration of bovine serum albumin. Thus the activation of HL by apoA-II appears to be specific and parallels closely the activation of LPL by apoC-II.

The modulation of LPL enzymic activity by apolipoprotein C-II has been actively investigated [4,5,20–22] and the physiological importance of this regulation is apparent from the low LPL activity and hypertriglyceridemia reported in patients with apoC-II deficiency [8]. The regulation of HL activity has received much less attention [3,14] and there has been, to our knowledge, no previous report of specific activators of this enzyme. It is known, however, that HL enzymic activity is enhanced by the addition of serum [14]. We have confirmed those findings and in these studies report that isolated apolipoprotein A-II activates HL. Under the assay conditions employed a 3-fold increase in HL activity was observed with the addition of μM quantities of apoA-II. The mechanism by which apoA-II activates HTGL enzymic activity in vitro remains to be determined. A comparison of the effects of apoA-II and apoC-II on HL and LPL enzymic activities suggests a similar mode of action for both apolipoproteins with regard to the in vitro hydrolysis of triolein emulsions.

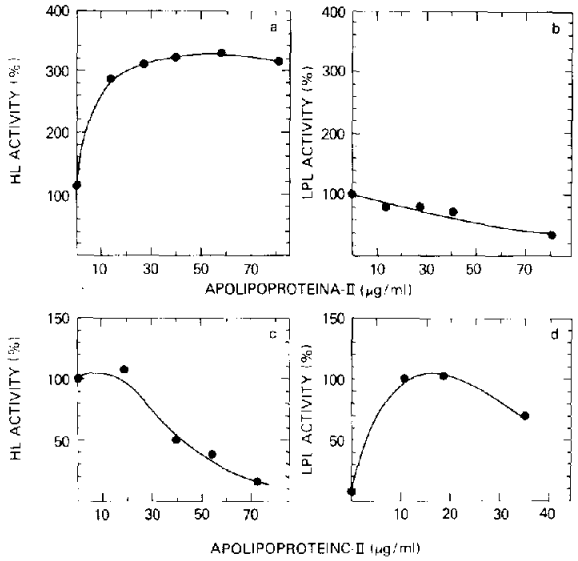

Fig.1. Effect of apolipoproteins A-II and C-II on HL and LPL enzymic activity. The enzymic activities of HL and LPL as a function of the concentration of apoA-II and apoC-II were studied: the effect of apoA-II on HL and LPL is given in (a) and (b), respectively and the effect of apoC-II on HL and LPL is given in panels (d) and (c) respectively. Assay conditions: 2 mM [^{3}H]triolein, 87 mM Tris–HCl, 2% BSA, 75 mM NaCl, 50 mM ammonium bicarbonate, 0.25 mM barbital (final pH 8.6) in a total volume of 200 μl. For the experiment shown in (b) apoC-II (10 μg/ml) was included in all assays.

Reports from several laboratories provide evidence that HL may play an important role in HDL metabolism. In the human, HDL_2 phospholipid and cholesterol concentrations are negatively correlated with

plasma HL enzymic activity [13]. HDL_2 cholesterol concentrations are also inversely correlated with HL enzymic activity in patients with primary biliary cirrhosis (unpublished). In the rat inhibition of HL enzymic activity after injection of a specific anti-HL antibody resulted in an increase of phospholipids and cholesterol in the HDL_2 density range (1.005–1.13 g/ml) [12,22] while HDL_3 lipid concentrations were reduced [12,22]. In addition, it has been reported in the rat that HL can hydrolyze HDL_2 phospholipids and the authors suggested that HL may be involved in the in vivo conversion of HDL_2 to HDL_3 [24]. ApoA-II is a major protein constituent of human plasma HDL and our results demonstrate that apoA-II in vitro activates HL enzymic activity. Our results are consistent with the concept that apoA-II increases the rate of HDL_2 metabolism through modulation of HL enzymic activity. The cofactor function of apoA-II is analogous to the cofactor functions of apoC-II for LPL in the metabolism of triglyceride-rich lipoproteins and apoA-I for lecithin cholesterol acyltransferase (LCAT) in the esterification of cholesterol (fig.2). Thus the enzymic activities of 3 enzymes involved in lipid metabolism

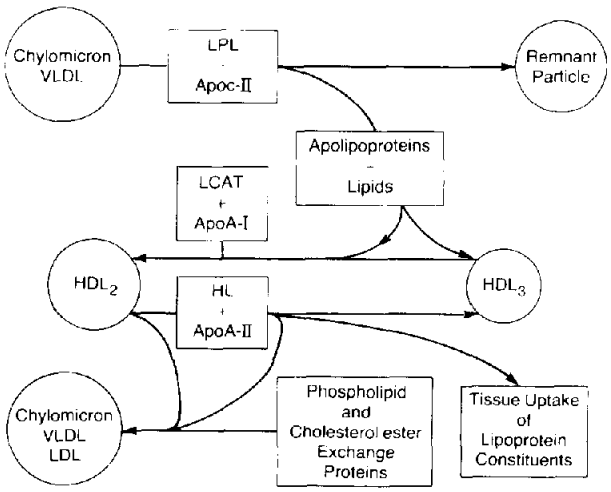

Fig.2. Current concepts of the regulation of HDL metabolism. Lipoprotein lipase (LPL) and its cofactor, apoC-II, catalyze the hydrolysis of chylomicron and VLDL triglycerides with the formation of remnant particles and the net transfer of apolipoproteins and lipids to HDL_3 and the conversion of HDL_3 to HDL_2. Within HDL lecithin cholesterol acyl transferase (LCAT) and its cofactor, apoA-I, catalyze the esterification of cholesterol to cholesterol esters. Hepatic lipase (HL) and its cofactor, apoA-II, catalyze the hydrolysis of lipids within HDL_2 with conversion of HDL_2 to HDL_3, the uptake of lipoprotein constituents by tissues and the net transfer of cholesterol ester and phospholipids to chylomicrons, VLDL, and LDL by exchange proteins.

(e.g., LPL, LCAT and HL) are regulated by apolipoproteins (e.g., apoC-II, apoA-I and apoA-II, respectively).

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